Derivatives of 6-Aminopenicillanic Acid. Part X.[1] A Non-enzymic Conversion of Benzylpenicillin into Semi-synthetic Penicillins

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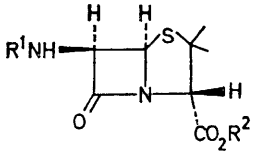
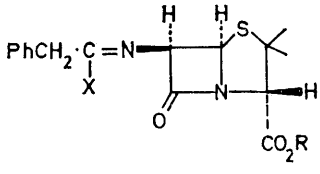
The carboxy-group of penicillin G is conveniently protected by treatment of the derived ethoxyformic anhydride with (*E*)-benzaldehyde oxime or (*E*)-2-furaldehyde oxime. Treatment with phosphorus pentachloride and *N*-methylmorpholine, followed by methanol, then gives the corresponding *O*-(6-aminopenicillanoyl)oxime. After introduction of any desired *N*-acyl substituent the carboxy-protecting group is removed, as the corresponding nitrile, by mild treatment with a nucleophile in the presence of anhydrous base.

PREPARATION of penicillins by direct fermentation is restricted to structures having side-chains derived from certain monosubstituted acetic acids. Thus, with the



(I)



(II)

exception of penicillin G (I; $R^1 = PhCH_2{\cdot}CO$, $R^2 = H$) and penicillin V (I; $R^1 = PhOCH_2{\cdot}CO$, $R^2 = H$), all penicillins in current clinical use are prepared by chemical acylation of 6-aminopenicillanic acid (I; $R^1 = R^2 = H$). Although 6-aminopenicillanic acid itself was originally obtained by fermentation,[2] removal of the phenylacetyl side-chain from penicillin G by the action of bacterial enzymes provides a more economical route.[3] Simple chemical hydrolysis of the phenylacetyl group is precluded because the more labile β-lactam amide link is attacked preferentially.

A different chemical deacylation procedure, originally developed in the cephalosporin series for preparing 7-aminocephalosporanic acid,[4] has also been applied [5]

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[4] B. Fechtig, H. Peter, H. Bickel, and E. Vischer, *Helv. Chim. Acta*, 1968, **51**, 1108.

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to the more labile penicillin G. Treatment with chlorotrimethylsilane gave the ester (I; $R^1 = PhCH_2CO$, $R^2 = SiMe_3$), which was then treated under strictly anhydrous conditions at about −10° with phosphorus pentachloride and pyridine, followed by methanol and then aqueous acid. Reaction probably proceeded *via* the imino-chloride (II; R = $SiMe_3$, X = Cl) and imidate (II; R = $SiMe_3$ or H, X = OMe), with final liberation of methyl phenylacetate, but the only material isolated was the end-product, 6-aminopenicillanic acid. Subsequently an improved version of the process was reported,[6] excellent yields being obtained by working at −40° and using dichloro(dimethyl)silane instead of chlorotrimethylsilane, and n-butanol instead of methanol. Meanwhile, having obtained only poor and erratic yields by the original procedure,[5] we sought to modify it by using a carboxy-protecting group which would remain in position until after the new *N*-acyl substituent had been introduced, but which could then be removed without disrupting the sensitive penicillin nucleus.

Conventional carboxy-protecting groups had various disadvantages for our purpose, but aromatic aldehyde oximes proved to be suitable reagents. Penicillin G and ethyl chloroformate gave the known mixed ethoxyformic anhydride (I; $R^1 = PhCH_2{\cdot}CO$, $R^2 = CO_2Et$) which, with (*E*)-benzaldehyde oxime or (*E*)-2-furaldehyde oxime, afforded the crystalline derivatives (I; $R^1 = PhCH_2{\cdot}CO$, $R^2 = N{:}CHPh$ or $N{:}CH{\cdot}C_4H_3O$). Treatment of the protected penicillin in methylene chloride or carbon tetrachloride with phosphorus pentachloride, preferably with *N*-methylmorpholine rather than pyridine as the base, resulted in a clean and smooth reaction without the need for exceptional precautions to exclude moisture. Methanol was then added, with cooling, followed, after 2 hr. at 0°, by water. Disappearance of the i.r. absorption at 1675 $cm.^{-1}$ indicated complete rupture of the secondary amide. Little of the resulting *O*-(6-aminopenicillanoyl) oxime (I; $R^1 = H$, $R^2 = N{:}CHPh$ or $N{:}CH{\cdot}C_4H_3O$) entered the strongly acidic aqueous layer, but the bulk was readily isolated as a crystalline salt by adding benzenesulphonic or toluene-*p*-sulphonic acid to the solvent phase. In a model experiment the isolated amine showed the same unexpected distribution between methylene dichloride and aqueous acid.

Acylation of the *O*-(6-aminopenicillanoyl) oxime with 3-*o*-chlorophenyl-5-methylisoxazole-4-carbonyl chloride, α-phenoxypropionyl chloride, or 2,6-dimethoxybenzoyl chloride in the presence of base then gave the crystalline carboxy-protected derivatives of cloxacillin, phenethicillin, and methicillin respectively.

The carboxy-protecting group was readily removed at room temperature by treatment with any of a number of nucleophiles in the presence of anhydrous base. Some nucleophilic reagents, such as sodium iodide or sodium thiocyanate, were conveniently employed in equimolecular quantity. In other cases it was more appropriate to employ a catalytic quantity of nucleophile, such as benzenethiol, together with a full equivalent of base. The sodium salt of the semi-synthetic penicillin generally crystallised from the reaction mixture, either directly or after addition of sodium (±)-2-ethylhexanoate. The overall yield of cloxacillin from penicillin G was 22%; no attempt was made to establish optimum conditions. In order to discover the fate of the oxime portion [7] we examined the by-products by i.r. spectroscopy and gas chromatography, and identified benzonitrile and furonitrile by comparison with authentic specimens.

EXPERIMENTAL

I.r. spectra were measured with a Perkin-Elmer 137 spectrophotometer (Nujol) and n.m.r. spectra were recorded with a Varian A-60 instrument, with dimethyl sulphoxide as solvent and tetramethylsilane as internal reference.

O-(6-*Phenylacetamidopenicillanoyl*) *Oximes*.—Penicillin G potassium salt (3·72 g., 0·01 mole) suspended in dry acetone (30 ml.) was cooled to −5° and treated with ethyl chloroformate (0·96 ml.) and pyridine (1 drop). The mixture was stirred at −5° for 30 min., treated with (*E*)-benzaldehyde oxime [8] (b.p. 122—124° at 12 mm., m.p. 32°; 1·2 g., 0·01 mole) in dry acetone (7 ml.), stirred for 2 hr. more without cooling, and filtered through kieselguhr. The filtrate was evaporated *in vacuo* and the residue was dissolved in ethyl acetate (30 ml.), then washed with successive 10 ml. portions of water, sodium hydrogen carbonate, and water again. The ethyl acetate solution was dried ($MgSO_4$), concentrated to small volume, and diluted with dry ether. Crystals of O-(6-*phenylacetamidopenicillanoyl*)*benzaldehyde oxime* (1·93 g.) were collected, washed with ether, and dried *in vacuo*; m.p. 140—142°, $\nu_{max.}$ 3380 (NH), 1795 (β-lactam C=O), 1770 (ester C=O), and 1680 (CO·NH) $cm.^{-1}$, δ 1·52 and 1·70 (6H, CMe_2), 3·60 (2H, CH_2), 4·64 (1H, C-3 H), 5·62 (2H, β-lactam H), 7·31 (5H, benzenoid H), 7·67 (5H, benzenoid H), and 8·82 (1H, N=CH−) p.p.m. (Found: C, 62·9; H, 5·6; N, 9·3; S, 7·2. $C_{23}H_{23}N_3O_4S$ requires C, 63·1; H, 5·3; N, 9·6; S, 7·3%).

Use of (*E*)-2-furaldehyde oxime [9] (m.p. 72—74° from benzene–light petroleum) instead of benzaldehyde oxime similarly gave O-(6-*phenylacetamidopenicillanoyl*)-2-*furaldehyde oxime* as crystals (from acetone–ether), m.p. 126—128°, $\nu_{max.}$ 3400 (NH), 1810 (β-lactam C=O), 1780 (ester C=O), and 1700 (CO·NH) $cm.^{-1}$, δ 1·54 and 1·71 (6H, CMe_2), 3·65 (2H, CH_2), 4·66 (1H, C-3 H), 5·67 (2H, β-lactam H), 6·70, 7·16, and 7·95 (3H, furan H), 7·32 (5H, benzenoid H), and 8·70 (1H, CH=N−) p.p.m. (Found: C, 59·5; H, 4·9; N, 9·8; S, 7·6. $C_{21}H_{21}N_3O_5S$ requires C, 59·0; H, 5·0; N, 9·8; S, 7·5%).

O-(6-*Aminopenicillanoyl*) *Oximes*.—(*a*) A solution of *O*-(6-phenylacetamidopenicillanoyl)benzaldehyde oxime (8·76 g.) in dry carbon tetrachloride (100 ml.) was cooled to −25°, treated with *N*-methylmorpholine (4·5 ml.), and stirred while a solution of phosphorus pentachloride (4·8 g.) in dry carbon tetrachloride (80 ml.) was added during 10 min.

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The mixture was stirred for 30 min. while the temperature rose to 0°, then re-cooled to −25° and treated with *N*-methylmorpholine (4·5 ml.) in dry methanol (160 ml.). After stirring for 2 hr. at 0°, water (300 ml.) was added and the pH of the aqueous phase was raised from 1·1 to 6·4 by addition of sodium hydroxide solution. The pale yellow solvent layer was separated, washed, dried, treated with benzenesulphonic acid monohydrate (3·5 g.) in acetone (25 ml.), and diluted to faint turbidity with dry ether. Next morning crystals of O-(6-*aminopenicillanoyl*)*benzaldehyde oxime benzenesulphonate* (8·7 g.) were collected, washed with dry ether, and dried *in vacuo*; m.p. 148—149° (decomp.), $\nu_{max.}$ 1800 (β-lactam C=O) and 1770 (ester C=O) cm.$^{-1}$, δ 1·57 and 1·71 (6H, CMe_2), 4·80 (1H, C-3 H), 5·18 and 5·68 (2H, β-lactam H), 7·40 (5H, benzenoid H), 7·69 (5H, benzenoid H), and 8·82 (1H, CH=N−) p.p.m. (Found: C, 52·5; H, 4·9; N, 8·4; S, 13·5. $C_{21}H_{23}N_3O_6S_2$ requires C, 52·8; H, 4·9; N, 8·8; S, 13·4%).

(*b*) The experiment was repeated with methylene chloride instead of carbon tetrachloride and toluene-*p*-sulphonic acid instead of benzenesulphonic acid. The salt did not crystallise directly, so the solvents were removed *in vacuo* and the residue was triturated with ethyl acetate to give O-(6-*aminopenicillanoyl*)*benzaldehyde oxime toluene*-p-*sulphonate* (62%), m.p. 153° (decomp.), $\nu_{max.}$ 1780 (β-lactam C=O), 1760 (ester C=O), and 1165 (SO_3) cm.$^{-1}$ (Found: C, 53·2; H, 5·1; N, 8·3; S, 13·1. $C_{22}H_{25}N_3O_6S_2$ requires C, 53·7; H, 5·1; N, 8·5; S, 13·0%).

(*c*) *O*-(6-Phenylacetamidopenicillanoyl)-2-furaldehyde oxime (4·27 g.) was treated as in (*b*) and the methylene chloride solution of the amine was treated with benzenesulphonic acid (1·76 g.) in dry acetone (10 ml.) followed by dry ether to the point of faint turbidity. The resulting crystals of O-(6-*aminopenicillanoyl*)-2-*furaldehyde oxime benzenesulphonate* (40%) had m.p. 149—150° (decomp.), $\nu_{max.}$ 1780 (β-lactam C=O), 1760 (ester C=O), and 1146 (SO_3) cm.$^{-1}$, δ 1·52 and 1·70 (6H, CMe_2), 4·75 (1H, C-3 H), 5·14 and 5·64 (2H, β-lactam H), 6·70, 7·18, and 7·97 (3H, furan H), 7·51 (5H, benzenoid H), and 8·75 (1H, CH=N−) p.p.m. (Found: C, 48·4; H, 4·5; N, 8·6; S, 13·6. $C_{19}H_{21}N_3O_7S_2$ requires C, 48·8; H, 4·5; N, 9·0; S, 13·7%).

N-*Acylation of* O-(6-*Aminopenicillanoyl*) *Oximes*.—(*a*) An ice-cold solution of *O*-(6-aminopenicillanoyl)benzaldehyde oxime toluene-*p*-sulphonate (4·91 g., 0·01 mole) in dry acetone (30 ml.) and triethylamine (2·8 ml.) was treated with 3-*o*-chlorophenyl-5-methylisoxazole-4-carbonyl chloride (2·56 g., 0·01 mole) in dry acetone (10 ml.) and stirred at 0° for 1 hr. The mixture was filtered through kieselguhr and the filtrate was evaporated *in vacuo*. The residue was dissolved in ethyl acetate (50 ml.) and washed with N-hydrochloric acid (10 ml.) followed by water (10 ml.); the resulting solution was dried ($MgSO_4$), concentrated under reduced pressure, and diluted with dry ether (3 volumes). Next morning the crystals were collected, washed with ether, and dried *in vacuo* to give O-[6-(3-o-*chlorophenyl*-5-*methylisoxazol*-4-*ylcarboxamido*)*penicillanoyl*]*benzaldehyde oxime* (3·8 g.), m.p. 144—146° (decomp.) (from acetone–ether), $\nu_{max.}$ 3400 (NH), 1795 (β-lactam C=O), and 1680 (CO·NH) cm.$^{-1}$, δ 1·53 and 1·60 (6H, CMe_2), 2·72 (3H, 5-CH_3), 4·68 (1H, C-3 H), 5·69 (2H, β-lactam H), 7·60 (5H, benzenoid H), 7·79 (4H, benzenoid H), and 8·84 (1H, N=CH) p.p.m. (Found: C, 57·7; H, 4·4; Cl, 7·0; N, 10·3; S, 6·1. $C_{26}H_{23}ClN_4O_5S$ requires C, 57·9; H, 4·3; Cl, 6·6; N, 10·4; S, 6·0%).

(*b*) Similar acylation of *O*-(6-aminopenicillanoyl)-2-furaldehyde oxime benzenesulphonate with α-phenoxypropionyl chloride gave O-[6-(α-*phenoxypropionamido*)*penicillanoyl*]-2-*furaldehyde oxime* (51%), m.p. 128—134°, $\nu_{max.}$ 3400 (NH), 1800 (β-lactam C=O), 1756 (ester C=O), and 1690 (CO·NH) cm.$^{-1}$, δ 1·41 and 1·53 (3H, CH_3), 1·50 and 1·62 (6H, CMe_2), 4·68 (1H, C-3 H), 4·93 (1H, −CH−), 5·60 (2H, β-lactam H), 6·72, 7·19, and 7·96 (3H, furan H), 7·01 (5H, benzenoid H), and 8·72 (1H, N=CH−) p.p.m. (Found: C, 57·6; H, 5·1; N, 9·3; S, 7·2. $C_{22}H_{23}N_3O_6S$ requires C, 57·7; H, 5·1; N, 9·2; S, 7·0%).

(*c*) Repetition of experiment (*b*) with 2,6-dimethoxybenzoyl chloride as the acylating agent gave O-[6-(2,6-*dimethoxybenzamido*)*penicillanoyl*]-2-*furaldehyde oxime* (53%), m.p. 144—146°, $\nu_{max.}$ 3330 (NH), 1810 (β-lactam C=O), 1780 (ester C=O), and 1670 (CO·NH) cm.$^{-1}$, δ 1·52 and 1·68 (6H, CMe_2), 3·77 [6H, 2,6-$(OMe)_2$], 4·57 (1H, C-3 H), 5·71 (2H, β-lactam H), 6·70, 7·18, and 7·98 (3H, furan H), 6·75 (3H, benzenoid H), and 8·74 (1H, N=CH−) p.p.m. (Found: C, 55·6; H, 5·2; N, 8·5; S, 6·8. $C_{22}H_{23}N_3O_7S$ requires C, 55·8; H, 4·9; N, 8·9; S, 6·8%).

Cloxacillin.—A solution of *O*-[6-(3-*o*-chlorophenyl-5-methylisoxazol-4-ylcarboxamido)penicillanoyl]benzaldehyde oxime (0·54 g., 0·001 mole) in dimethylformamide (1 ml.) was treated with benzenethiol (1 drop) and triethylamine (0·28 ml.). After 2 hr. at room temperature the solution was treated with 1·67N-sodium (±)-2-ethylhexanoate in isobutyl methyl ketone (0·6 ml., 0·001 mole) followed by (±)-2-ethylhexanoic acid (0·16 ml., 0·001 mole), and evaporated *in vacuo*. The residual yellow oil was dissolved in water-saturated isobutyl methyl ketone (40 ml.) and diluted with the dry solvent (60 ml.), whereupon it gradually deposited crystals of 6-(3-*o*-chlorophenyl-5-methylisoxazol-4-ylcarboxamido)penicillanic acid sodium salt monohydrate (0·3 g., 63%), identical with an authentic specimen [10] (i.r. spectra and biochromatograms).

Phenethicillin.— *O*-[6-(α-Phenoxypropionamido)penicillanoyl]-2-furaldehyde oxime was treated as in the previous experiment except that the quantity of triethylamine was halved and the (±)-2-ethylhexanoic acid was omitted. The yield of crystalline 6-(α-phenoxypropionamido)penicillanic acid sodium salt was 60%.

Methicillin.—(*a*) *O*-[6-(2,6-Dimethoxybenzamido)penicillanoyl]-2-furaldehyde oxime (2·4 g., 0·005 mole) in dry acetone (20 ml.) was treated with sodium iodide (0·75 g., 0·005 mole) in dry acetone (10 ml.), followed by triethylamine (0·7 ml.). After 1 hr. water (5 drops) was added and the mixture was stirred for 4 hr. while crystallisation took place. 6-(2,6-Dimethoxybenzamido)penicillanic acid sodium salt monohydrate (1·7 g., 85%) was collected, washed with acetone, and dried *in vacuo*. The product was identical (i.r. spectra and biochromatograms) with an authentic sample.[11]

(*b*) When sodium thiocyanate was used in place of sodium iodide the yield of sodium methicillin was 61%.

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